Emirates Journal of Food and Agriculture. 2023. 35(12): 1-8
doi: 10.9755/ejfa.2023.3185
http://www.ejfa.me/

RESEARCH ARTICLE

Physicochemical, morphological and functional characteristics of starch isolated from *Quercus ilex* and *Quercus coccifera*

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ABSTRACT

The present research work examined and compared the morphological, physicochemical and functional characteristics extracted from two oak varietiesgrowing inTunisia. Among the findings, it was observed that *Quercus ilex*'s starch granules had the the hightest value of lipid and phosphor content. When observed under scanning electron microscopy, both *Quercus ilex* and *Quercus coccifera* starch granules exhibited various shapes such as ovoid, spherical, elliptical and irregular, with diameters ranging from 4 to 25 μm. As for the X-ray diffraction patterns, acorn starch from both varieties displayed an A-type pattern. Comparing the properties, *Quercus coccifera* acorn starch exhibited higher values for the light transmittance values, the solubility, the swelling power and the gelatinization temperatures than *Quercus ilex* acorn starch. However, the transition enthalpy of *Quercus coccifera* acorn starch was the lowest among the studied parameters. These results indicate that these properties are significantly ($p<0.05$) different depending on the oak variety. Thus, the starches of *Quercus ilex* and *Quercus coccifera* could play different roles in various industrial applications.

Keywords: acorn; functional properties starch; *Quercus*; physicochemical properties

INTRODUCTION

Oak is an abundant species in Mediterranean forests (Sheffer, 2011), and researchers from all around the world have conducted several studies to explore the potential of oak acorn and their marketability, especiallyin the food industry. Acorns are rich in starch, fat, minerals, such as Ca, P, K, and Mg, as well as unsaturated fatty acids i.e., oleic acid, (Lassoued et al., 2022), and vitamins, mainly A and E (Vinha et al., 2016; Salajpal et al., 2008). They also contain various biologically active compounds, namely tannins, phenolic acids, and flavonoids, which are essential for maintaining appropriate antioxidant levels in the human diet to keep appropriate antioxidant levels (Vinha et al., 2016). Flour made from acorns is a popular product, and it is important to assess the physical and chemical qualities of these new flour products before their development.

The flour swelling power is closely associated with the characteristics of starch content (Blazek and Copeland, 2008). The biosynthesis of starch comes in granular form, and the size, shape and crystal structure of these granules depend on their botanical origin (Xu et al., 2014). In its granular form, starch is a plentiful, renewable, and affordable ingredient that serves as a thickening, gelling and binding agents in foods. It can be studied in various ways, either as a physical entity with a wide range of shapes and sizes (Singh et al., 2003), or as a chemical entity made up primarily of glucose polymers with a semi-crystalline structure (Tetlow and Bertoft, 2020). Besides, starch can be used for species identification in botany (Kamba, 2008).

Starch has applications beyond the food industry and is used in non-food industrial sectors like paper industry, pharmaceutical industry, cosmetics and textiles (Vinha et al., 2016). It has recently been a material of interest for

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Received: 20 July 2023; **Accepted:** 09 November 2023

the development of renewable plastics and biodegradable materials (Avérous and Halley, 2009), and biofuels (Balat and Balat, 2009). The functional properties of native starchas swelling, retrogradation, gelatinization, pasting, and susceptibility to enzymatic digestion are all directly linked to structural features. However, acorn starch lacks systematic information when compared to other potato, corn, and wheat starches. As it is the case with the frequently used extraction processes, starch features are influenced by both starch sources and starch history itself (Wichmann et al., 2007). As indicated in the available the literature, the selection of the starch isolation methods is likely to be determined by the attributes of the raw material and the intended end-use applications.

In this study, the alkaline procedure was used to extract the starches from oak acorns. Many researchers have investigated alkaline extraction to yield high-purity extracts(Boudries et al., 2009; Wang et al., 2000). As previously suggested (Lassoued et al., 2022) the starch was extracted from both *Quercus coccifera* and *Quercus ilex*, and the ratios of amylose/amylopectine were determined using the identical samples used in this study. As far as we know, the functional attributes as well as the physicochemical properties, namely the distribution of the particle size have not been explored for *Quercus coccifera* acorn starch. The goal was, therefore, to determine whether these two sources of starch share similarities with each other and with starch from other sources, and to assess their pertinence in various fields.

Fig 1. *Quercus coccifera* (A) and *Quercus ilex* (B) fruits.

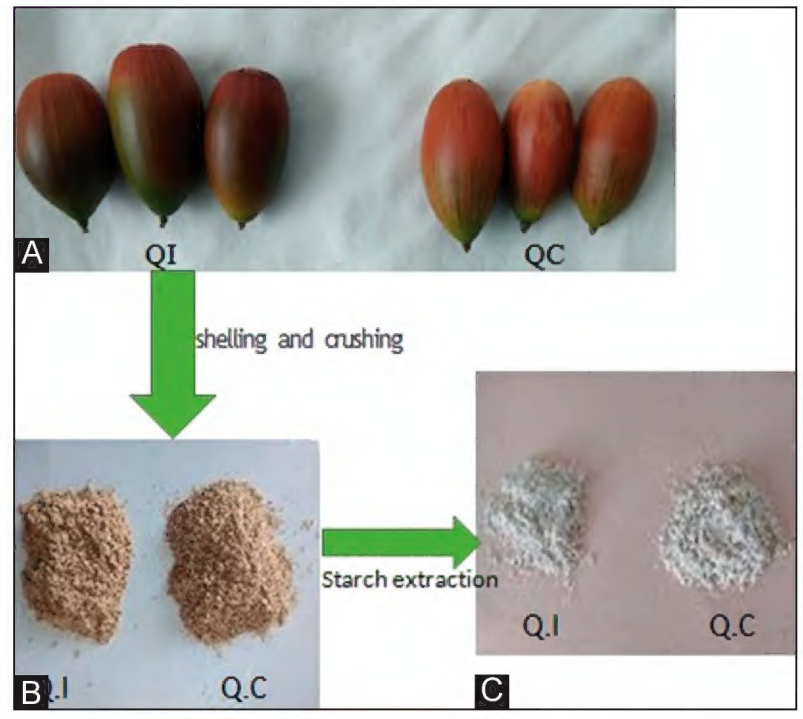

Fig 2. Schematic diagram of starch extraction of QI: *Quercus ilex* and QC: *Quercus coccifera*, A: acorn; B: flour and C: starch.

MATERIALS AND METHODS

Plant materials

The collection of the acorn fruits of *Quercus coccifera* (QC) and *Quercus ilex* (QI) (Fig. 1) was realized from Nabeul, a town in northern Tunisia, during the harvest season. The acorns were shelled, dried in the open air at room temperature and then crushed using a grinding machine.

Starch isolation

As can be seen from Fig. 2, the isolation of the starch from acorn flour was realized by the alkaline by Nwokocha and Williams's method (2011). The starch that was acquired was allowed to dry naturally in the air and then kept in airtight containers for storage.

Proximate chemical composition

While the measurement of the moisture content was conducted following ISO 6496 (1999) method, that of the protein content from the total nitrogen (N × 6.25) was carried out by means of Kjeldahl method (ISO 5 983 (2009)). The concentrations of lipid, ash and phosphor were established using ISO 6492 (1999), ISO 5984 (2003) and ISO 6491(1998) methods, respectively.

Scanning electron microscopy

The acorn starch samples were examined using a scanning electron microscope (FEI/Quanta 650 FEG) with field effect (SEM FEG) equipped with field effect (SEM FEG) and energy-dispersive X-ray microanalysis (EDS). The acorn starch samples were equally distributed and placed on stub with The samples were evenly spread and affixed to a stub using double-sided adhesive tape. An acceleration voltage of 10 kV was applied for the analysis.

Color measurement

Color analysis was conducted using a Minolta Chroma Meter CR-300 (Osaka, Japan), and the classification was conducted according to CIELAB system. Colorimeter measurements of the starch samples were expressed as L* (lightness), a* (red/green opponent colors), and b* (yellow/blue opponent colors).

X-ray diffraction

The analysis of theX-ray diffraction was performed in an X-ray diffractometer (Bruker AXS/D8 ADVANCE

A25) running at forty kV and 200 mA. The scanning of the sample was scanned through the 2θ from 3 to 50 ° at a speed of 8°/min and a scan step of 0.02. The relative crystallinity degree was calculeted using the equations below:

$$Relative\ crystallinity\ \%\ Ac = Ac/(Ac + AA) \times 100 \quad (1)$$

where *Ac* is the crystalline area and *AA* is the amorphous area on the X-ray diffractogram.

Fourier transform infrared spectroscopy

The PerkinElmer/Frontier IR/FIR spectrophotometer instrument was employed to record the infrared spectra of oak gland starch. The recorded spectra were executed at 4000-400 cm^{-1} with a resolution of 4.0 cm^{-1}. It is to be mentioned that starch samples were mixed with KBr.

Thermogravimetric analysis

The Quercus starch samples were analyzed by the SETARAM SETSYS Evolution 1750 type thermobalance according to the method described by Boukhelkhal and Moulai-Mostefa (2017).

Differential scanning calorimetry analysis (DSC)

The thermalproperties of starch samples were determined using a model Mettler Toledo/DSC 3 apparatus, following the procedure outlined by Boukhelkhal & Moulai-Mostefa (2017). Key parameters, including onset temperature (To), the peak temperature (Tp), the conclusion temperature (Tc) and the gelatinization enthalpy (ΔH) were obtained from the obtained thermogram.

Swelling power and solubility

The swelling power and solubility were identified in triplicate following the method described by Boukhelkhal & Moulai-Mostefa (2017) with slight modifications. The starch solution of 2% (starch/distilled water) was supplemented into acentrifuge tube, and heated for thirty minutes in a thermostat water bath at various temperatures (60, 70, 80, 90°C) constant shaking. After cooling to ambient temperature, the samples were centrifuged at 4500 rpm for 20 min. The supernatant was parted and dried until it reached a consistent weight (m_s). The calculation of the swelling power and solubility was conducted using the equations below:

$$Solubility(g/g\ dry\ starch) = \frac{m_s}{m_0} \quad (2)$$

$$Swelling\ power\ (g/g\ dry\ starch) = \frac{m_1}{m_0\left(1 - solubility\right)} \quad (3)$$

where:

m_0: the amount of dry starch (g)

m_1: the weight of swollen starch granules (g)

Light transmittance measurement (Turbidity)

The measurement of turbidity of the 1% starch suspensions was realized following the method of Zang et al. (2020) using a UV-visible spectrophotometer. Light transmission measurment was taken at 620 nm against a water blank.

Statistical analysis

All data represent the means of at least 3 different determinations. For statistical analysis, the Statistic® vs 6 software was used. The findingswere subjected to an analysis of variance, and the Fisher LSD test was used to determine the significance of the discrepancies between the means. Thelevel of significance for all statistical testswas set at 95%.

RESULTS AND DISCUSSION

Proximate chemical composition

Table 1 shows the data on the chemical composition properties of starches isolated from acorns of QI and QC. The starch's average moisture percentage varies from 16.1% to 16.3%, the protein percentage from 2.93% to 3.02%, and the fat percentage from 2% to 2.8%. These starches have a moisture content that is within the moisture limit (< 20%) recommended for native starches (Boukhelkhal and Moulai-Mostefa, 2017) which allows for a desirable shelf life. When compared to rice and maize starch, both cultivars have a high protein level between 0.35% and 0.52% (Abida et al., 2014). The lipid concentrations range from 2 to 2.8%, with *Quercus ilex* having the significantly highest lipid content ($p<0.05$) (2.8%). These findings are higher than those observed for holm oak starch (0.23 to 0.63%) by (Boukhelkhal and Moulai-Mostefa, 2017). Phosphorus represents one of the non-carbohydrate components found in starches, which influences their functional characteristics. Phosphorus content of *Quercus ilex* and *Quercus coccifera* acorns is higher than that of corn starch (0.003%) and lower than that of potato starch (0.09%) (Chaves-Morillo and Mejía-España, 2023). Growing conditions, temperature, and storage time have all been shown to alter the phosphorus level and form of potato starch(Siddiqui et al., 2021).

Morphological properties

The morphology of acorn starch granules is shown in Fig. 3. SEM images reveal that the starch granules have a

Table 1: Chemical composition of starches isolated from *Quercus coccifera* and *Quercus ilex*

Sample	Moisture (%)	Protein (%)	Lipid (%)	Phosphor (%)
Quercus coccifera	16.3±0.2[a]	2.93±0.04[a]	2.00±0.2[b]	0.01±0.001[b]
Quercus ilex	16.1±0.2[a]	3.02±0.04[a]	2.8±0.2[a]	0.02±0.001[a]

Values are means of 3 Samples±Standard Deviations (SD). Different letters (a, b) in the same row express significantly different results $p<0.05$ between *Quercus coccifera and Quercus ilex*

wide range of shapes (ovoid, spherical, irregular, and large elliptical shapes). Large and irregular granules were found in *Quercus coccifera*, which are identical to those reported in potato starch granules (Singh et al., 2003).The acorn starch granules had a smooth surface, with an average granule size varying between 4 and 22 µm for *Quercus ilex* and between 6 and 25 µm for *Quercus coccifera*.Such values are higher than those of *Quercus rotundifolia* and *Quercus suber* starches,which vary from 5 to 17 µm (Correia et al., 2021). However, they are lower than those of the starch of *Quercus leucotrichophora* that are in the range of 21 and 59 µm (Pan et al., 2014). The botanical origin and changes in climatic conditions are primarily responsible for starch particle size variation (Pascoal et al., 2013). Furthermore, granule size is connected to physicochemical qualities, including swelling power and gelatinization (Singh et al., 2003).

Color measurement

Table 2 displays the values of the color in the CILAB system (L*, a*, b*) for acorn starch samples. The samples of acorn starch *Quercus cocifera* and *Quercus ilex* indicate substantially different L* values, suggesting that the starch of *Quercus coccifera* has the greatest whiteness. The low values of a* for all acorn starch samples suggested a low level of tone saturation. Acorn starch has been shown in certain investigations to be yellow or beige (Correia et al., 2009), which accords well with the results of the present study (the b* value ranges from 17.46 to 17.62). Pigments like carotene and other polyphenolic compounds, which are present in the acorn could be accredited to the starch quality (Abegunde et al., 2013). Based on these findings, acorn starches could be used in baked goods that are not affected by the color of the starch such as cookies.

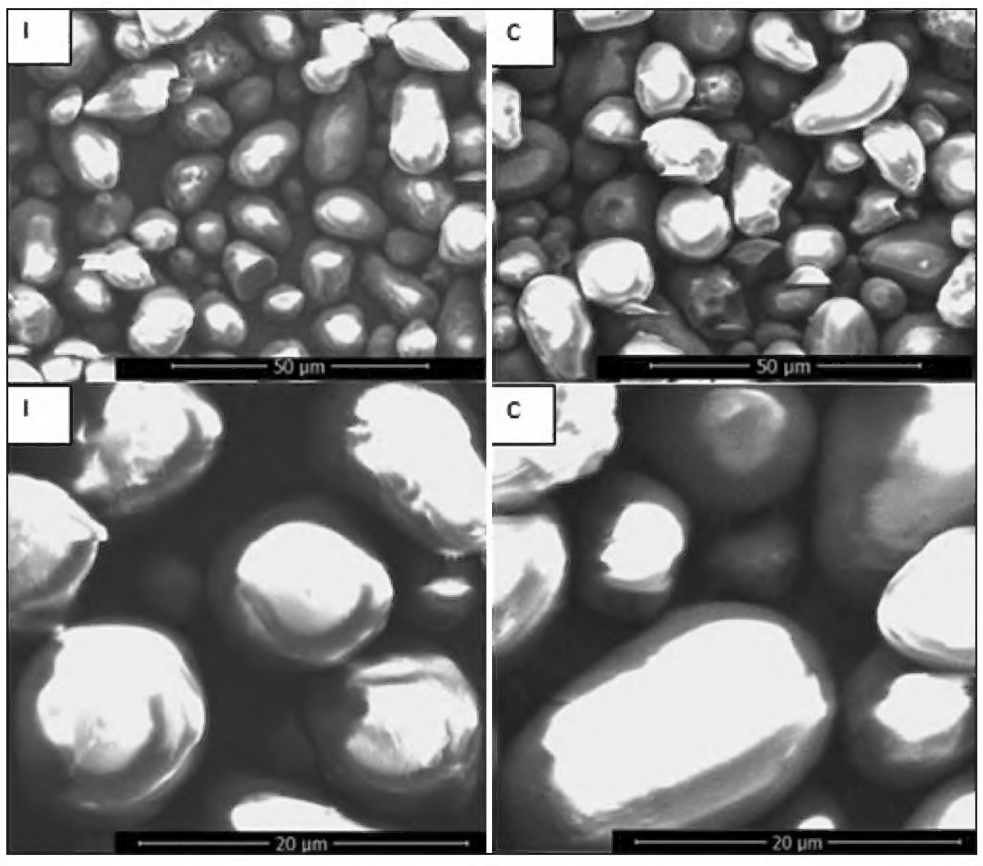

Fig 3. SEM images of acorn starches (I: *Quercus ilex*; c: *Quercus coccifera*); ×1000, ×3000.

Crystalline properties

According to their diffractogram, native starches are categorized into four classes (A, B, C and V) Sun, 2020). Fig. 4 (a) demonstrates that the starch from the acorns of *Quercus ilex* and *Quercus coccifera*has have a type A X-ray diffraction pattern. The acorn starch has strong reflections at 15°, 17° and 22.5, which is in accordance with the results reported by Li et al, (2015) for *Quercus glandulifera* Bl. According to Stevenson et al. (2006), the degree of relative crystallinity of starch from *Quercus ilex* and *Quercus coccifera* was proven to be higher than that *Quercus palustris Muenchh* (22.3%). As exhibited in Table 2, the intensity of the distinctive peaks and the relative crystallinity of the starch acorn*Quercus ilex* (40.45%) are much higher than the starch of the acorns of *Quercus coccifera* (31.07 %). Taib and Bouyazza (2021) reported that the crystallinity degree of acorn starch were in the range of 22.3 and 47.8%. The crystalline fraction (19%) of sweet potato starch was lower than that of acorn starch (Liu et al., 2019). Its poor crystallinity could be explained by the lack of a crystalline amylopectin phase in sweet potato starch (Liu et al., 2019). Furthermore, the genotypic, agronomic, and growth circumstances had the greatest influence on the diffraction pattern and degree of crystallinity of starch granules (Zhang et al., 2020; Deng et al., 2020).

FT-IR spectra analysis

Fig. 4 (b) presents the FTIR spectra of the extracted oak acorn starches of *Quercus ilex* and *Quercus coccifera*. The spectra for the two kinds are comparable, indicating the absorption bands that characterize the starch. The spectra indicate an extremely large band at 3296 cm^{-1} emanating from the vibration stretching of the O-H bpnd, and a band at 2926 cm^{-1} induced by the C-H bond stretching. The significant peaks detected at 1641 cm^{-1} were described to the scissor vibration of the OH bonds of the absorbed water molecules (H_2O). The peaks of 523–1140 cm^{-1} are generally accredited to the stretching of the C–O bonds corresponding to the deformation vibration of the connections of the C–O–C and C–O– H glycoside bonds.

Table 2: Color, the relative crystallinity and the turbidity of acorn starch

Sample	The color parameters			Relative crystallinity [%]	Turbidity
	L*[%]	a*[%]	b*[%]		
Quercus coccifera	77.2±0.36^{a}	1.42±0.03^{b}	17.62±0.15^{a}	31.07±1.12^{b}	3.2±0.13^{a}
Quercus ilex	74.79±0.23^{b}	1.89±0.02^{a}	17.46±0.12^{a}	40.45±1.56^{a}	1.55±0.11^{b}

Values are means of 3 samples±Standard Deviations. Different letters (a, b) in the same row express significantly different results p<0.05 between *Quercus coccifera and Quercus ilex*

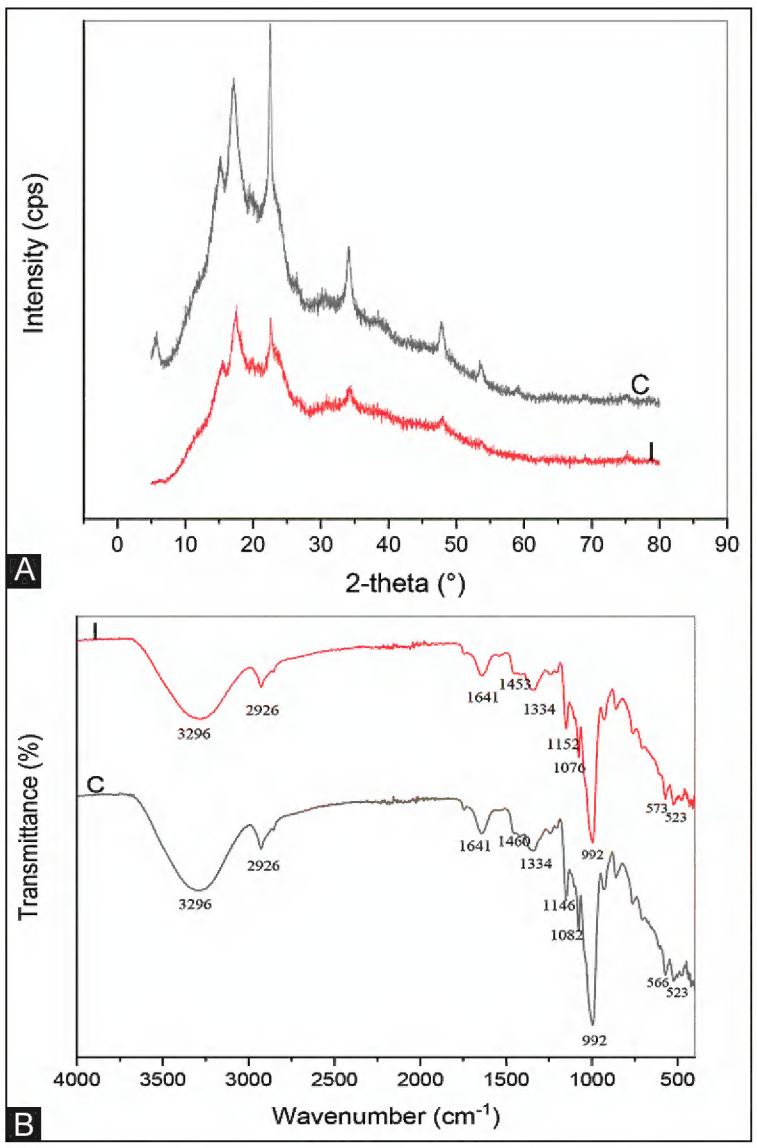

Fig 4. (A) X-ray diffraction patterns and (B) FT-IR spectrum of acorn starches(I: *Quercus ilex*; C: *Quercus coccifera*).

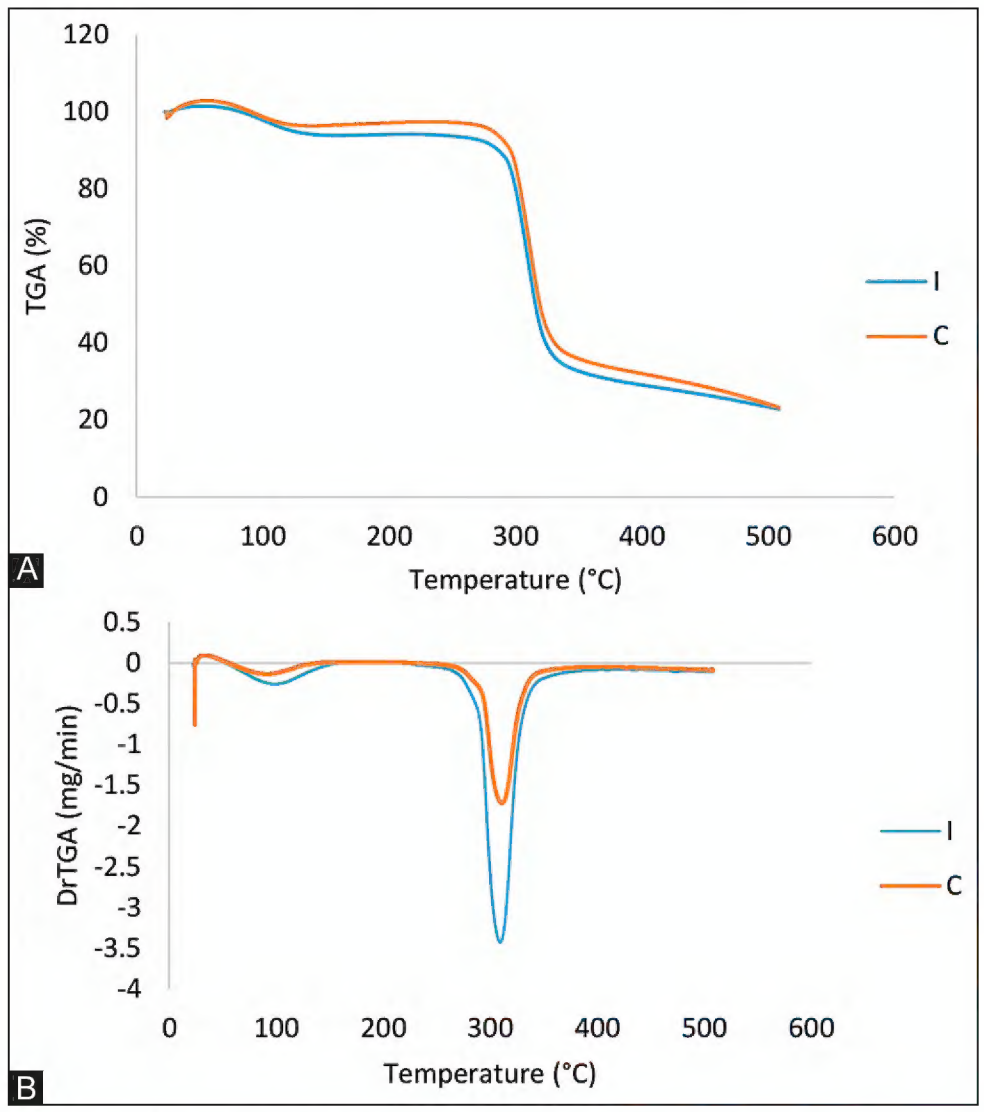

Fig 5. (A) TGA curves of acorn starches,(B) DTGA curves of acorn starches. (C; *Quercus coccifera*, I; *Quercus ilex*).

The obtained findings are in good agreement with those observed for holm oak starch (Boukhelkhal & Moulai-Mostefa, 2017). Indeed, Irinislimane and Belhaneche-Bensemra (2017) have reported that the FTIR spectra of extracted oak acorn and potato starches exhibit similar absorption bands describing the starch.

Thermal properties

The technique of thermogravimetric analysis (TGA) has been broadly utilized in the examination of polymeric systems. This analytical technique measures the weight loss of a sample in a given environment as a function of temperature to evaluate its thermal stability and volatile component content. The first TGA derivative, DTG, offers information on the rate of starch breakdown relative to other TGA derivatives. The maximal weight loss rate is shown by the DTG curve's biggest peak. The thermogravimetric analysis (TGA) curves of acorn starch of *Quercus ilex* and *Quercus coccifera* are presented in Fig. 5 (a), revealing three phases of mass loss. The first phase corresponding to a small endothermic peak in the DTGA curve is attributed to dehydration, ranging between 20°C and 130°C and (Fig. 5 (b)). The percentage of mass loss in this step is determined depending on the sample's moisture content. The most significant weight loss befell between 300°C and 345°C which corresponds to the decomposition of organic materials (amylose and amylopectin) and terminates at around 345°C. The creation of ash relates to the third phase of degradation, which was relatively sluggish. The degradation of these polymers is broken down into stages in Table 3.

Gelatinization is a physical parameter that indicates the structural features of starch molecules is gelatinization. When exposed to high temperatures, starch granules swell irrevocably due to water absorption, resulting in an augmentation of viscosity.

Table 4 shows the DSC-derived thermal characteristics of acorn starch. The temperature transition (To, Tp and Tc) and ΔH of the starch varied significantly according to the varieties of acorns ($p < 0.05$). The obtained temperatures at the onset of gelatinization (59.75°C and 62.75°C for *Quercus coccifera* and *Quercus ilex*, respectively) accord well with those reported for acorn starches in two other research works (58.4°C and 60.9°C for *Quercus suber* (Correia et al., 2013) and *Quercus branti* (Molavi et al., 2018). The main gelatinization temperature (Tp) was approximately 63.35 °C and 65.74 °C for *Quercus ilex* and *Quercus coccifrera* respectively, which is lower than that of the oak acorn (88.1°C) (Boukhelkhal and Moulai-Mostefa, 2017). Although the final gelatinization temperature (Tc) value is in the range of 66.12°C and 72.93°C, it is substantially lower than the high value of holm oak grown in Algeria (98°C -120°C) (Boukhelkhal and Moulai-Mostefa, 2017). The discrepancies in To, and gelatinization temperature array in starches from diverse cultivars are likely to be ascribed to the differences in the degree of crystallinity. According

Table 3: Thermogravimetric (TG) and differential thermogravimetric (DTGA) analysisresults

Sample	TG results			DTGA results Tp (°C)
	Number of decomposition step	Temperature range (°C)	Weighht loss (%)	
Quercus coccifera	1st	85±0.79-122±2.33	4±0.23	94.24±0.77
	2nd	276±3.26-335±3.99	57.3±0.69	315.14±3.13
	3rd	340±2.96-500±5.23	14±0.36	
Quercus ilex	1st	80±0.69-139±3.31	6±0.31	100.75±1.19
	2nd	278±2.63-338±4.14	55.5±1.66	309.3±2.67
	3rd	340±3.97-500±4.88	14±0.56	

Values are means 3 samples±Standard Deviations (SD) of three determinations. Tp: peak temperature

Table 4: Thermal properties of holm oak starches

Sample	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Quercus coccifera	59,75±0.35[b]	65,74±0.48[a]	72.93±0.33[a]	5,71±0.17[b]
Quercus ilex	62,75±0.51[a]	63,35±0.29[b]	66.12±0.52[b]	7,04±0.22[a]

Values are means of 3 samples±Standard Deviations. Different letters (a, b) in the same row express significantly different results $p<0.05$ between *Quercus coccifera and Quercus ilex.* T_0 :onset temperature, T_P : peak temperature, T_C : conclusion temperature, ΔH : enthalpy of gelatinization

to Othman et al. (2011), crystalline structures are more thermally stable than amorphous structures since they experience thermal deterioration at a higher temperature compared to the amorphous structure. The high transition temperatures have been proven to emanate from a high crystallinity degree of (Askadskii et al., 2014).

The gelatinization enthalpy of acorn starch (5.71J/g and 7.04J/g for *Quercus ilex* and *Quercus coccifrera* respectively) was lesser than that of *Quercus palustris* Muench (20.8 J/g) (Stevenson et al., 2006),indicating that the preliminary gelatinization step necessitates less energy. Research works have reported that ΔH emanates essentially from the rupture of the double helices rather than the long-distance disturbance of crystallinity (Chen et al., 2016). As a result, the gelatinization capabilities of different starches vary, which could be attributable to a variety factors such as the mineral composition, the amylose/amylopectin ratio, the shape and size of the starch granules, and the molecular architecture of the starch crystalline region (Pérez-Pacheco et al., 2014). The ΔH of *Quercus ilex* starch shows that double helices were markedly disrupted during gelatinization greater than in *Quercus coccifera* starches. This leads to the conclusion that to accomplish gelatinization, more energy would be required to disrupt the intermolecular interactions in *Quercus ilex* starch granules.

Swelling power and solubility

According to Correia et al. (2009), the swelling power and solubility values reflect the interactions between amylose/amylopectin or between amylopectin molecules. When heated in excess water, the starch granules enlarge and their crystalline structure is shattered. During this process, water molecules form hydrogen bonds with the bare hydroxyl groups of amylose and amylopectin, increasing solubility and swelling power (Li et al., 2013). Fig. 6 shows that the

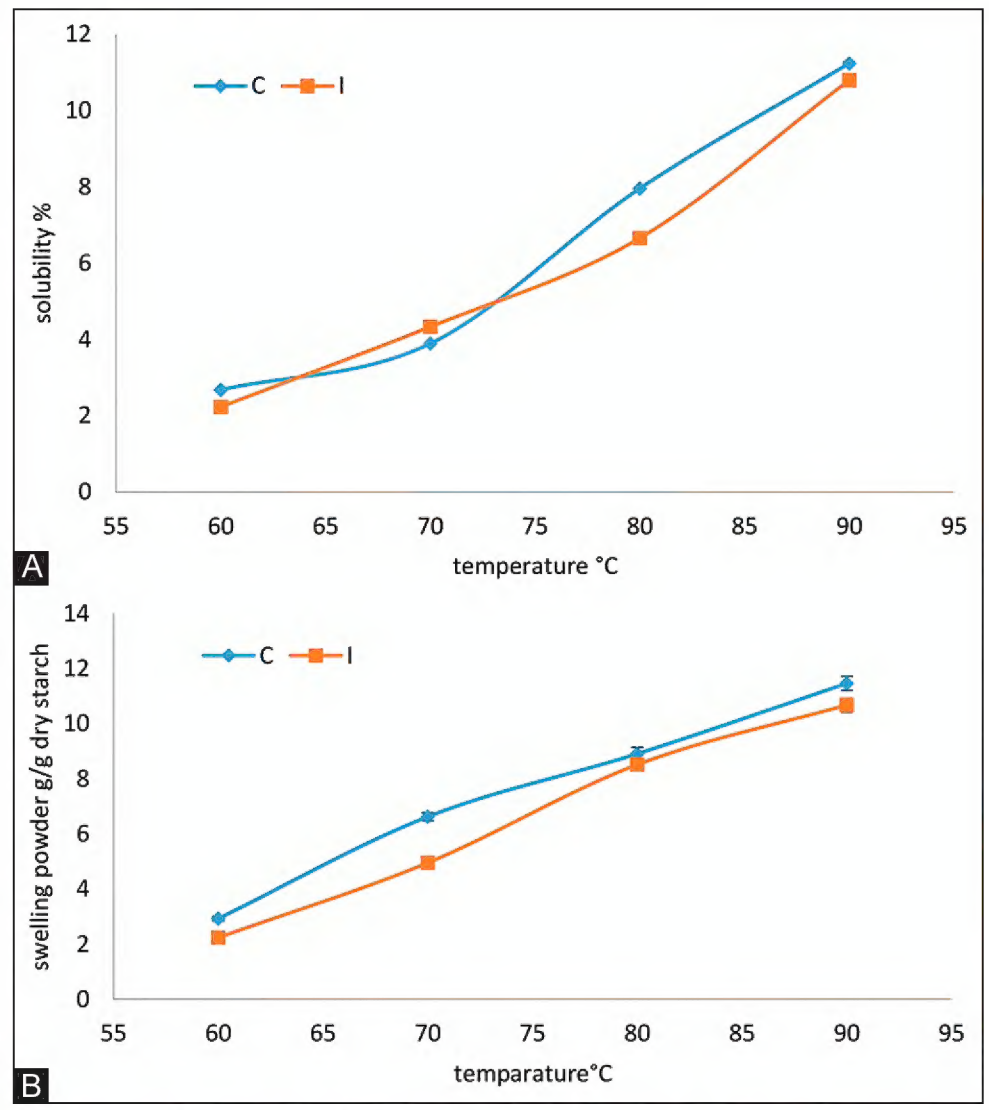

Fig 6. (A) Solubility of the varieties and (B) Swelling power of acorn starches (C; *Quercus coccifera*, I; *Quercus ilex*).

swelling power and solubility of starches from*Quercus ilex* and *Quercus coccifera* increase with temperature. The crystalline molecular structure of starch can be disrupted during high-temperature cooking, enhancing absorption capacity and solubility (Singh et al., 2003). *Quercus coccifera* starch showed higher swelling power and solubility values than *Quecus ilex* starch, which could be explained by the variances in morphological structure of starch granules (Singh et al., 2003).

Kaur et al. (2002) have discovered that potato starches with large and irregular granules have a higher swelling power. Large and irregular granules may aid in the immobilization of the starch material within the granule. According to Ratnayake et al. (2002), the amylose content has the greatest influence on solubility, while amylopectin has the biggest influence on swelling power. The variations in amylose and amylopectin ratios, molecular weight, and morphological structure of starch granules such as granule size may also contribute to

modifications in starch solubility and swelling powers (Li et al., 2013; Wani et al., 2012). As for Singh et al. (2003), they have proposed that phosphate group content is a major aspect affecting the swelling power of starch. Phospholipids in starch tend to be complex with amylose and long-branched chains of amylopectin, resulting in restricted swelling. Furthermore, the existence of lipids instarch may reduce the ability of starch granules to expand (Singh et al., 2003).

Turbidity analysis

Translucency is an important visual feature of starch that reflects the acceptance of starch-based products. The turbidity values of *Quercus ilex* and *Quercus coccifera* acorn starch are displayed in Table 2. The turbidity of *Quercus ilex* starch is significantly lower ($p< 0.05$) than that of *Quercus cocciera* starch. The interaction of various factors such as granule swelling, amylase and amylopectin chain length as well as phosphorus and lipid contents are mainly responsible for turbidity in native starches (Sandhu and Singh., 2007). According to Gomand et al. (2010), potato starch with higher phosphate monoester content produces pasta with higher transmittance than other starches.

CONCLUSION

The current studyexamined the structural features, physicochemical and functional characteristics of starches isolated from two varieties of oak acorns.The obtained findings revealed that the levels of moisture, protein, fat and phosphor ranged from 16.1 to 16.3, 2.93 to 3.02, 2 to 2.8, and from 0.01 to 0.02 %, respectively. The gelatinization temperatures as well as theswelling power of*Quercus cocifera* starches were found to be higher than those of *Quercus ilex* starch. Besides, the *Quercus ilex* starch were characterised by the highest values of relative crystallinity and enthalpy of gelatinization. The obtained results have proven that swelling power and starch solubility increase with temperature, with *Quercus ilex* starch exhibiting the lowest values. The light transmittance of the starches extracted from these two varieties of oak acorns varied significantly ($p < 0.05$).

The results obtained not only contribute to a deeper understanding of the structural and functional characteristics of oak acorn starch, but also offers valuable insights for potential chemical, physical, and enzymatic adjustments of starch. Besides, the obtained results establishthat acorn oak starch can be asubstitute resource for broader applications in the food industry.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support of the Tunisian Ministry of Higher Education and Scientific Research. They also extend their thanks to the staff of Composite Materials and Clay Minirales Laboratory.

Authors' contributions

The experiments were carried out by Rabeb Lassoued, Wafa Najar Benahmed, Aymen Benhmed and Mounir Ferhi. Rabeb Lassoued analyzed the data and wrote the manuscript. The work was completed under the direction of Manef Abderrabba and Jamel Mejri. Finally, the manuscript was revised and approved by Jamel Mejri.

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